

Calibration of a System for the Computer-Assisted Operation of a Small Animal Inhalation Facility

by Ethard W. Van Stee* and Michael P. Moorman*

The initialization of chambers in the computer-assisted inhalation facility at the National Institute of Environmental Health Sciences (1) includes a series of operations that we call "characterization." Characterization consists of two parts, the first of which is one of the topics of this report. In the first part of the characterization the mathematical relationship between the concentration of the chemical of interest and the output of the analyzer is approximated. This amounts to establishing a standard against which subsequent, daily calibrations can be compared. The second part of the characterization represents a wholly automatic operation in which certain dynamic characteristics of the system are quantified.

A daily calibration is performed at the beginning of each day of chamber operation after the system has been characterized. The daily calibration data are checked against the characterization standard. The conversion equation for the daily operation of the chamber is derived from the daily calibration data combined with the characterization data.

An equation that converts the output of the analyzer to units of concentration of the chemical of interest is at the heart of the computer-assisted monitoring and control system for our inhalation facility. The equation is derived from a calibration procedure that is conducted prior to starting each day's chamber operation. Quality control requires that, in addition to having a daily calibration of the system, a standard of reference be available against which each day's calibration data can be checked. This practice provides protection against the introduction of spurious calibration data on a daily basis, as well as providing a means for the detection of longer term drift.

A simulation program was written to model the computations prior to installation of the task as part of the operating system for the facility. The program allowed repeated characterization and calibration operations to be simulated in a relatively short period for the purpose of refining certain details governing the operation of the task. The simulated raw data were based on actual performance records of several chamber operators.

Chamber Characterization Summary

Prior to starting a new series of exposures a procedure is performed that we call "characterization." Characterization consists of two parts: establishing a calibration standard to which subsequent daily calibration data are compared and quantifying dynamic properties of the system.

Figure 1 represents a general outline of the sequence of computations from which the calibra-

tion standard is derived. The analyzer is purged with zero air and the electrometer is zeroed. Resetting zero is not permitted during the following operation. Aliquots of standard chemical are injected sequentially into the calibration loop in increments that have been computed to yield six concentrations in order of increasing concentration. The operator pushes a button in between each injection to signal the system to read the analyzer. After each sixth injection the operator purges the calibration loop with zero air and the sequential injection process is repeated. The cycle is repeated 10 times during which the computer logs the equivalent of 10, consecutive six-point

*National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.

calibrations consisting of zero and six concentrations.

The initial array of concentration-analyzer response data is represented in the upper left hand quadrant of Figure 1. Having provisionally accepted the first 10 rows of data (moving counterclockwise in Figure 1), the computer performs a polynomial fitting and stepwise reduction operation on the data (2). The polynomial that we fit represents a "no-intercept model" (3), i.e., it does not include a zero-order (constant) term, and thus, in a graphical sense, forces a plot of the function through the origin. The algorithm first fits a fourth-order polynomial to the data and then tests the significance of the estimated coefficients after computing their respective partial F ratios.

The user will have provided the computer systems with critical values for these F ratios against which the computed, partial F ratios will be compared. Selection of the critical values was based on the outcome of a computer simulation (q.v.).

The polynomial reduction process begins by testing the coefficient of the fourth-order term. If the F ratio for the coefficient exceeds the critical value the associated term is accepted as the significant term of maximum degree. If the value of the F ratio is less than the critical value, the coefficient of the third-order term is tested. The process is continued until the first significant coefficient is encountered. Once the polynomial of a degree represented by the significant coefficient of highest order has been fitted to the original raw data, the machine computes an F ratio for variation among the rows (4). As in the case of the critical values for the F ratios for the coefficients, the user will have supplied the system with a critical value for an F ratio for variation among the rows that is determined by his needs.

If the F ratio for variation among the rows is less than the critical value, the 10 rows are accepted. If the F ratio is greater than the critical value a polynomial is refitted to the data (Fig. 1, lower left center) and the operator is instructed to make six additional, incremental injections of chemical into the calibration loop, thus creating an 11th row of data.

Moving counterclockwise in Figure 1, an analysis of homogeneity is performed on all 11 combinations of 10 rows using the degree of polynomial that was most recently computed. Again the F ratios are tested against the critical values and the combination yielding the smallest F -ratio is retained. The computational sequence continues until 10 rows of homogeneous data have been

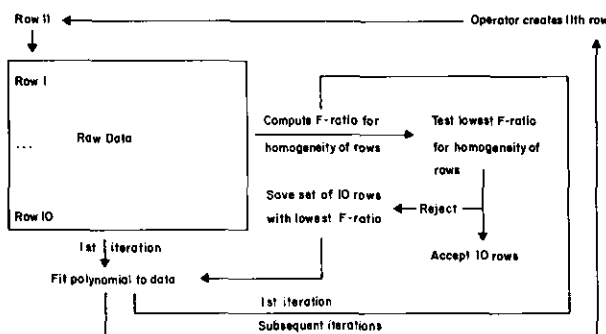


FIGURE 1. This is an illustration of the algorithm that is used to create an initial 10 rows of homogeneous calibration data. Subsequent daily calibration data are compared to the 10 rows of data for the purpose of generating the daily control equation (see text for details).

acquired or until the computational process is overridden.

Characterization Algorithm and Computations

The operator executes a series of sequential injections of standard chemical into the calibration loop of the analyzer that results in the filling of a matrix of raw data.

Analyzer reading

Row 1	(0)	Y(1,1)	Y(1,6)
Row 2	(0)
Row 3	(0)
Row 4	(0)
Row 5	(0)
Row 6	(0)
Row 7	(0)
Row 8	(0)
Row 9	(0)
Row 10	(0)	Y(10,1)	Y(10,6)
	X(0)	X(1)	X(2)	X(3)	X(4)	X(5)	X(6)
							PPM

(1)

Immediately following the 60th injection the polynomial fitting routine begins execution. The $X'X$ matrix (2) is computed from the 60 values of x , i.e., the concentrations in ppm of standard chemical that were associated with each analyzer reading. The values of x have been computed previously in response to the interactive query session. The elements of the b vector are the coefficients to be estimated. The elements of the $X'Y$ vector are the sums of cross-products.

$$\begin{array}{cccccc}
& [X'X] & & [b] & & [X'Y] \\
\Sigma X^2 & \Sigma X^3 & \Sigma X^4 & \Sigma X^5 & b_1 & \Sigma XY \\
\Sigma X^3 & \Sigma X^4 & \Sigma X^5 & \Sigma X^6 & b_2 & = \Sigma X^2Y \\
\Sigma X^4 & \Sigma X^5 & \Sigma X^6 & \Sigma X^7 & b_3 & \Sigma X^3Y \\
\Sigma X^5 & \Sigma X^6 & \Sigma X^7 & \Sigma X^8 & b_4 & \Sigma X^4Y
\end{array} \quad (2)$$

The coefficients are estimated by inverting the $X'X$ -matrix and multiplying by the $X'Y$ -vector (5).

Thus

$$X'X\hat{b} = X'Y \quad (3)$$

becomes

$$\hat{b} = (X'X)^{-1}X'Y \quad (4)$$

where \hat{b} is the estimate of b .

Examination of an analysis of variance table for a regression analysis (Table 1) provides an illustration of the logic behind the sequence of computations that is used to select the highest polynomial of "best fit," i.e., the polynomial of highest order with a statistically significant coefficient for the highest order. Note that at this stage the regression computation results in the fitting of a single curve common to all of the data points by the method of least squares.

The total sum of squares (SST) is divided into the fraction that is determined by regression (RegSS), i.e., determined by the dependence of y on x , and the remainder, or residual, ResSS. Since the test for the significance of each term is based on a ratio of the associated partial RegMS and the ResMS it is necessary to go through a series of computations that will result in a partitioning of the RegSS.

The computations are based on Eqs. (3) and (4) and are performed on the matrix and vectors of Eq. (2) or on matrices and vectors that are modified from Eq. (2). RegSS (4), RegSS (3), RegSS (2), and RegSS(1) [see Eqs. (5), (6), (8), (10)] are the regression sums of squares that are associated with polynomials of fourth, third, second, and first order, respectively. Note that the summations that are indicated or used are taken over the entire 60 data points.

Computation of the Sum of Squares for Regression for a Fourth-Order Polynomial

$$\begin{aligned}
SSReg(4) = & \hat{b}_1\Sigma XY + \hat{b}_2\Sigma X^2Y + \hat{b}_3\Sigma X^3Y + \\
& \hat{b}_4\Sigma X^4Y - (\Sigma Y)^2/60 \quad (5)
\end{aligned}$$

Table 1.

Source of variation	Degrees of freedom	Sums of squares	Mean squares
Regression	4	SSReg	MSReg
X term	1		
X ² term	1		
X ³ term	1		
X ⁴ term	1		
Residual	55	SSRes	MSRes
Total	59	SST	

The coefficients $\hat{b}_1, \dots, \hat{b}_4$ are estimated exactly as indicated in Eqs. (2), (3) and (4), i.e., the $X'X$ -matrix is inverted and multiplied by the $X'Y$ -vector.

Computation of the Sum of Squares for Regression for a Third-Order Polynomial

$$\begin{aligned}
SSReg(3) = & \hat{b}_1\Sigma XY + \hat{b}_2\Sigma X^2Y \\
& + \hat{b}_3\Sigma X^3Y - (\Sigma Y)^2/60 \quad (6)
\end{aligned}$$

The coefficients $\hat{b}_1, \dots, \hat{b}_3$ are estimated from a matrix and vectors that are modified from Eq. (2), i.e., the last row and column of the $X'X$ -matrix and the last elements of the b - and $X'Y$ -vectors, respectively, are eliminated. The estimates of b_1, \dots, b_3 , are the solutions to Eq. (7):

$$\begin{array}{cccccc}
& [X'X] & & [b] & & [X'Y] \\
\Sigma X^2 & \Sigma X^3 & \Sigma X^4 & b_1 & & \Sigma XY \\
\Sigma X^3 & \Sigma X^4 & \Sigma X^5 & b_2 & = & \Sigma X^2Y \\
\Sigma X^4 & \Sigma X^5 & \Sigma X^6 & b_3 & & \Sigma X^3Y
\end{array} \quad (7)$$

Computation of the Sums of Squares for Regression for a Second-Order Polynomial

$$SSReg(2) = \hat{b}_1\Sigma XY + \hat{b}_2\Sigma X^2Y - (\Sigma Y)^2/60 \quad (8)$$

The coefficients \hat{b}_1 and \hat{b}_2 are estimated from a matrix and vectors that are modified from Eq. (7), i.e., the last row and column of the $X'X$ -matrix and the last elements of the b - and $X'Y$ vectors, respectively, are eliminated. The estimates of b_1 and b_2 are the solutions to Eq. (9):

$$\begin{array}{cccccc}
& [X'X] & & [b] & & [X'Y] \\
\Sigma X^2 & \Sigma X^3 & b_1 & & & \Sigma XY \\
\Sigma X^3 & \Sigma X^4 & b_2 & = & & \Sigma X^2Y
\end{array} \quad (9)$$

Table 2.

Source of variation	Regression sum of squares	Source of added variation	Regression sum of squares
X	RegSS(1)	X_2	RegSS(1)
$X + X^2$	RegSS(2)	X_3	RegSS(2)-RegSS(1)
$X + X^2 + X^3$	RegSS(3)	X_3	RegSS(3)-RegSS(2)
$X + X^2 + X^3 + X^4$	RegSS(4)	X_4	RegSS(4)-RegSS(3)

Computation of the Sums of Squares for Regression for First-Order Polynomial

$$\text{SSReg}(1) = \hat{b}_1 \Sigma XY - (\Sigma Y)^2/60 \quad (10)$$

The coefficient \hat{b}_1 is estimated according to

$$\hat{b}_1 = \Sigma XY / \Sigma X^2 \quad (11)$$

in which ΣXY and ΣX^2 are the first elements of the $X'Y$ and $X'X$ matrices of Eq. (2), respectively.

Degrees of Freedom

The number of degrees of freedom associated with RegSS is equal to the arithmetic value of the exponent of the term of highest order, initially, 4. One degree of freedom is associated with each X term of the polynomial. The number of degrees of freedom that is associated with SST is equal to one fewer than the total number of data points, i.e., $60 - 1 = 59$. The number of degrees of freedom for ResSS is the difference between that of the total sum of squares (SST) and that of the regression sum of squares (RegSS).

Partition of the Regression Sum of Squares

The regression sum of squares is partitioned into components that are associated with the respective X -terms. The RegSS that were computed in Eqs. (5), (6), (8) and (10) are tabulated to the left in Table 2. Consecutive differences are tabulated to the right in Table 2.

Total Sum of Squares

The total sum of squares [Eq. (12)] is equal to the sum of the squares of each analyzer reading, the $Y(i,j)$ of (1), minus the square of the sum of the $Y(i,j)$ divided by the total number of readings, i.e.,

$$\text{SST} = \Sigma Y^2 - (\Sigma Y)^2/60 \quad (12)$$

Table 3.

Residual mean square, ResMS	To obtain F ratio for
$\text{ResMS}(1) = [\text{SST}-\text{RegSS}(1)]/58$	Coefficient of X term
$\text{ResMS}(2) = [\text{SST}-\text{RegSS}(2)]/57$	Coefficient of X^2 term
$\text{ResMS}(3) = [\text{SST}-\text{RegSS}(3)]/56$	Coefficient of X^3 term
$\text{ResMS}(4) = [\text{SST}-\text{RegSS}(4)]/55$	Coefficient of X^4 term

Table 4.

F Ratio
$\text{RegSS}(1)/\text{ResMS}(1)$
$[\text{RegSS}(2)-\text{RegSS}(1)]/\text{ResMS}(2)$
$[\text{RegSS}(3)-\text{RegSS}(2)]/\text{ResMS}(3)$
$[\text{RegSS}(4)-\text{RegSS}(3)]/\text{ResMS}(4)$

Test of Significance of Coefficients of Polynomial

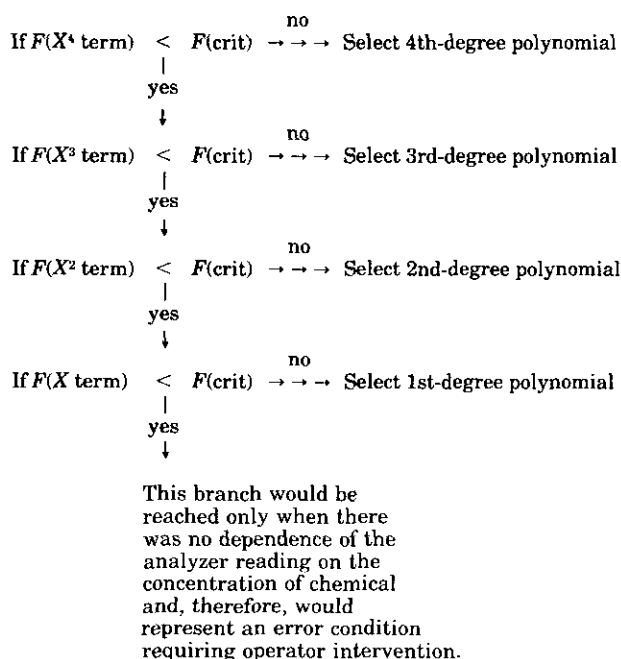
The coefficients are tested by computing F ratios for each one. These are the partial F ratios of the regression analysis and they are obtained by dividing the mean square associated with the difference between the two, consecutive polynomial models by the residual mean square that is associated with the polynomial model of higher order (Table 3).

In our case the F ratios that correspond to the respective coefficients are computed by dividing the appropriate differences between regression sums of squares (RegSS) by the appropriate residual mean squares (ResMS) (Table 4). When computing the numerators of the F ratios, the difference between the two RegSS is divided by the appropriate degrees of freedom which, in this special case, equals one.

The user must select criteria for the rejection of coefficients. The criteria may be critical values for the F ratio that have been selected from tables of F ratios that are commonly found in statistics books. If the computed value for the F ratio that corresponds to the coefficient of the term of highest order is greater than the critical value the term can be considered to be significant and, therefore, should be retained. Alternatively, if the computed F ratio is less than the critical value, the term can be rejected. In the testing of

statistical hypotheses critical values for the F ratios are selected on the basis of the probability of committing a Type I error, i.e., in this case, retaining the term when the coefficient is, in fact, not significantly different from zero.

A particular characteristic of the application of a stepwise regression analysis to our data influences our selection of the critical values for the F ratios. Somewhat overfitting the data incurs little penalty so long as a monotonic relationship between concentration and response is preserved. Therefore, if we err in retaining a nonsignificant term (Type I error) we may not encounter any problem beyond that of the extra computation that would be associated with the unnecessary terms. Eliminating a significant term (Type II error) could be more serious since the shape of the concentration-analyzer response curve could be seriously affected. Thus, since the two error probabilities are inversely related, selecting critical values of F corresponding to probabilities (of committing a Type I error) of 0.05–0.10 will be less likely to result in rejecting significant terms than if the critical values are selected to correspond to probabilities of 0.01 or less. The critical values of F that are selected are associated with 1 (numerator) and 55, 56, 57 or 58 (denominator) degrees of freedom, depending on whether the order of the polynomial of higher order is 4, 3, 2 or 1. The stepwise elimination is performed according to algorithm (13).



(13)

Following selection of a degree of polynomial of less than fourth-order, the algorithm returns to Eq. (7) in the case of third-order, Eq. (9) in the case of second-order or Eq. (11) in the case of first-order, and re-estimates the coefficients.

Building a Homogeneous Characterization Data Base

Estimates of the coefficients of the polynomial that best fits the 10 rows of calibration data have been determined by this point and have been provisionally accepted only for the purpose of finding what degree of polynomial to use. No attempt has yet been made to assess the homogeneity of those rows of data, each of which represents the equivalent of a six-point calibration. Conceptually, the algorithm has found the best-fitting curve that is common to all 60 data points.

The next step involves quantifying mathematically definable differences among the 10 rows of data with the aim of rejecting and replacing one or more rows of data that are unacceptable based on a defined set of criteria. Rejected rows of data are replaced with new data derived from additional injections that are performed by the operator. Presumably, better and better rows of data are accumulated until 10 are accepted. The basis of that sequence of computations is illustrated in the analysis of variance table (Table 5). Note that the summations that are indicated or used in Tables 5–7 and Eq. (14) are now taken over the 10 rows of data rather than over the 60 data points.

Table 5.

Source of variation	Regression SS
Common curve	RegSS(1,2,3 or 4) ^a
Among rows	$\Sigma \text{RegSS} - \text{RegSS}(1,2,3 \text{ or } 4)$
Pooled residual	ΣResSS

^aThe common curve represents the result of the computations that were described above.

Table 6.

Row	n	RegSS	ResSS
1	6	RegSS ^a	ResSS ^b
2	6	"	"
3	6	"	"
4	6	"	"
5	6	"	"
6	6	"	"
7	6	"	"
8	6	"	"
9	6	"	"
10	6	"	"
		ΣRegSS	$\Sigma \text{ResSS} = \text{Pooled residual SS}$

^aRegression SS computed for each row individually.

^bResidual SS computed for each row individually.

Table 7.

Source of variation	Degrees of Freedom			
	Order of polynomial selected			
	1st	2nd	3rd	4th
"Among rows" RegSS	10	20	30	40
Pooled residual SS ^a	40	30	20	10

^adf(pooled residual) = df(total) - df("among rows").

The homogeneity of the curves that represent the 10 rows of data varies as an inverse function of the ratio of the "among rows" regression mean square to the pooled residual mean square. Mean squares are obtained by dividing sums of squares by their corresponding degrees of freedom. The sums of squares for this computation are accumulated as illustrated in Table 6.

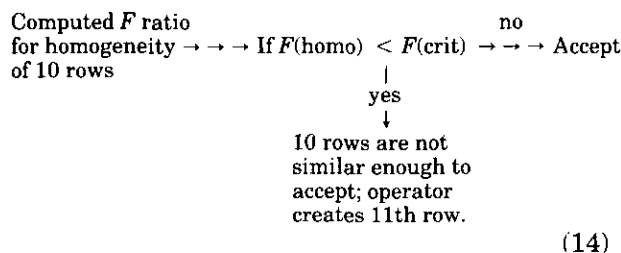
Calculation of the RegSS and ResSS for each row follows a computational scheme analogous to that of Eqs. (2) and (5) in the case of a fourth-order polynomial, Eqs. (6) and (7) in the case of a third-order polynomial, Eqs. (8) and (9) in the case of a second-order polynomial, and Eqs. (10) and (11) in the case of a first-order polynomial. Since the raw data for each computation consist of six pairs of concentration-response data from the analyzer, n equals 6 rather than 60 as in Eqs. (5), (6), (8) and (10). The "among rows" sum of squares in Table 5 is the arithmetic sum of the RegSS for the 10, individual curves minus the "common curve" RegSS, and the pooled residual sum of squares equals the arithmetic sum of the corresponding ResSS.

An F ratio for homogeneity of the 10 rows of data (lower center of Fig. 1) is obtained by dividing the "among lines" regression mean square by the pooled residual mean square. The mean squares are obtained from the sums of squares by dividing them by the appropriate degrees of freedom as tabulated in Table 7. The total degrees of freedom will be $10(n - 1) = 50$, where 10 is the number of rows. The degrees of freedom of the numerator will be the order of the polynomial (exponent of the term of highest order) times the number of rows minus one.

The user must select a critical value for use in algorithm (14) for comparison with the F ratio for homogeneity of the 10 rows of calibration data of which the characterization data matrix (1) is composed. The critical value should be selected to correspond to the order of the polynomial.

The null hypothesis states that the curves that are fitted to each of the rows are the same. Committing a Type I error would result in rejecting the hypothesis when it was true. The cost of

committing a Type I error is realized as a requirement on the part of the operator to create an 11th line of data, i.e., make six additional standard injections. The cost of committing a Type II error, rejecting the alternative hypothesis that the lines are not homogeneous when it is true, is in the magnitude of the standard error of the estimates of the coefficients that are used in judging the acceptability of subsequent daily calibration data. Because of the nature of the scheme that is employed for establishing the criterion of acceptance of the daily calibration data, the penalty for committing a Type I error is actually greater than the penalty for committing a Type II error. The greater the critical value of F , the lower the probability of committing a Type I error. Therefore, as a general recommendation, $F(\text{critical})$ should be selected to correspond to probabilities in the range of 0.05–0.01 (of committing a Type I error), although the actual values may be varied to suit the particular needs of the user.



When the first 10 rows of data are not homogeneous according to Eq. (14), the operator is instructed to create an 11th row of data. A variation of a previously described computational sequence is activated to select the 10 best of 11 rows of data. Essentially, the polynomial is refitted and then the algorithm loops back to Table 6 and proceeds to the point of the branch in Eq. (14) for 11 iterations, each of which includes a different sample of 10 rows. During each iteration an F ratio for homogeneity of rows is computed. The group of 10 rows with the lowest F ratio for homogeneity is then used for the subsequent calculations.

The decision-making process continues exactly as in Eq. (14) from this point. The operator is required to make successive series of six standard injections until 10 have been accumulated with an "among rows" F ratio that is less than the critical value, or until the process is manually overridden.

Evaluating Daily Calibration Data

The initialization that must be performed by the operator as part of the daily operation of the

system includes making six, standard, calibration injections, the results of which will be compared with the standard calibration (characterization) data. The maximum degree of the polynomial to be fitted to the calibration data has been fixed. The coefficients of the polynomial fitted to the daily 6-point calibration are determined according to Eqs. (2) and (5) in the case of a fourth order polynomial, Eqs. (6) and (7) in the case of a third-order polynomial, Eqs. (8) and (9) in the case of a second-order polynomial, or Eqs. (10) and (11) in the case of a first-order polynomial, except that n in the correction terms of Eqs. (5), (6), (8) and (10) equals 6 rather than 60.

The daily calibration is accepted or rejected according to a scheme based on weighted sums of standard, normal deviations of the estimates of the coefficients of the daily calibration data from the estimates of the coefficients of the 10 rows of characterization data. The standard errors of the estimates of the coefficients of the polynomial fitted to the characterization data are computed from the residual mean square (ResMS) and the inverse of $X'X$ according to Eq. (15) (6).

(1st diagonal element of $X'X^{-1}$)(ResMS) = s^2 for coefficient of X term
 (2nd diagonal element of $X'X^{-1}$)(ResMS) = s^2 for coefficient of X^2 term
 (3rd diagonal element of $X'X^{-1}$)(ResMS) = s^2 for coefficient of X^3 term
 (4th diagonal element of $X'X^{-1}$)(ResMS) = s^2 for coefficient of X^4 term

(15)

where

$$\text{ResMS} = (\text{SST} - \text{RegSS}) / (59 - m) \quad (16)$$

The regression sum of squares (RegSS) of Eq. (16) equals RegSS(m), where m is numerically equal to the value of the exponent of the significant term of highest order. The standard error of an estimate of a coefficients from the characterization data equals the square root of s^2 .

The sum of weighted standard normal deviations (SND) for the daily calibration is computed according to Eq. (17). To obtain the SND the absolute value of the difference between the estimate of the coefficient \hat{b}_m that was computed for the polynomial that was fitted to the characterization data, and the estimate of the coefficient \hat{b}'_m that was computed for the polynomial that was fitted to the daily calibration data, is divided by the standard error of the estimate of the coefficient se_b for the characterization data. This is done for each consecutive term of the polynomial. Each SND is multiplied by its corresponding weighting factor (W). The weighted SNDs are added together. The user provides the acceptance criterion to be used.

$$\begin{aligned} \Sigma \text{SND}_w = & W_1 \left(\frac{|\hat{b}_1 - \hat{b}'_1|}{se_{b_1}} \right) + W_2 \left(\frac{|\hat{b}_2 - \hat{b}'_2|}{se_{b_2}} \right) \\ & + W_3 \left(\frac{|\hat{b}_3 - \hat{b}'_3|}{se_{b_3}} \right) + W_4 \left(\frac{|\hat{b}_4 - \hat{b}'_4|}{se_{b_4}} \right) \end{aligned} \quad (17)$$

The reason for using a scheme of sums of weighted SNDs is rooted in the relationship between the operating requirements of the chamber control system and the general shape of the conversion equation. As stated earlier the equation must represent a monotonic relationship between concentration and analyzer response. When the control system is regulating the chamber concentration at midrange a linear approximation of the polynomial relationship is usually an accurate enough representation of the concentration-response relationship of the analyzer to permit satisfactory operation of the chamber. This may be all that is necessary when the chamber is to be run only at a constant, midrange concentration. However, when time-varying concentration profiles are to be run, it becomes imperative to account for the curvilinear response of the analyzer, especially at the lower and higher ends of the concentration-response curve. In our experience we have found that, in general, the magnitude of the weighting factor by which successive terms are multiplied varies inversely with the order of the term.

An example of an acceptance criterion that might be used in our implementation of the system would be to add 1.5 times the SND for the X term, 1 times the SND for the X^2 term, 0.5 times the SND for the X^3 term and 0.2 times the SND for the X^4 term. When this sum of weighted, standard normal deviations of the coefficients was greater than the user-defined acceptance criterion the daily calibration would be rejected and the operator would be required to perform a new series of six, standard injections. Obviously, the probability that the daily calibration will be accepted increases with the magnitude of the acceptance criterion. The user will select an acceptance criterion based on his own requirements.

An alternative and somewhat more straightforward approach to evaluating the daily calibration curve would be to follow the sequence of calculations that is described in Tables 5–7 and Eq. (14) for building a characterization data base and to

consider each daily calibration curve as an 11th row of data. Although this approach is somewhat simpler than the approach that involves the use of weighted SNDs it does not permit the user to weight the lower ordered coefficients.

Computing the Daily Control Equation

Each day new calibration data are compared with the 10 rows of characterization data (the standard calibration reference). After the daily calibration data have been accepted a polynomial is fitted to all data in the characterization data base plus all of the accumulated, accepted daily calibration data, to yield the control equation for operating the chamber for that day. It is important to restrict the acceptance test of the daily data to the characterization data only, since using the accumulating data base for this purpose would be accompanied by gradually decreasing values for the standard errors of the estimates of the coefficients which would have the practical consequence of making it increasingly difficult, day by day, to get a daily calibration accepted.

Computer Simulation

We used a computer to model the interaction between the operator and the calibration tasks from the computer-assistance software package. The simulation permitted us to evaluate some of the effects on overall accuracy of calibration both of operator performance and of different values assigned to constants that are used at various decision points. The simulation program was constructed by adding modules for control, report generation, and operator emulation to the computation modules from the characterization and calibration tasks.

The simulation program was initialized by entering various constants, data representing properties of the performance of a fictitious operator, analyzer transfer functions, critical values for variance ratios, report formats, number of "days" (number of repetitions of the daily calibration for each characterization) and the number of times that the characterization-calibration cycle was to be repeated (equivalent to the number of new exposure experiments to be simulated). The end

product of a simulation run was a tabulation of characterization and characterization results. Printouts of raw data and the results of critical, intermediate calculations were also available.

The operator emulation module produced normally distributed random numbers that conformed to a polynomial representation of the analyzer transfer function. The effects of operator performance were demonstrated by varying the standard deviation of the random numbers. The ability to recover properly various transfer functions was assessed by modifying the coefficients of the generation polynomial.

Typical values for the standard deviations describing operator performance were obtained by analyzing calibration data from several operators using different experimental set-ups. The coefficients for a typical analyzer transfer function were obtained by performing a 24-point calibration using carbon tetrachloride.

Simulations were run first using the numbers that reflected actual, typical performance of operator and analyzer. The various critical constants were then adjusted iteratively in order to achieve reasonable performance. Once this was achieved the numbers that represented operator and analyzer performance were modified to model the effects of changing operators and experimental set-ups.

The authors wish to thank Richard A. Sloane for his technical assistance and Earl Linthicum for the graphics. A special debt is owed to Dr. Gregg Dinse of the Biometry and Risk Assessment Program of NIEHS who reviewed the statistical methods and provided many constructive suggestions.

REFERENCES

1. Van Stee, E. W., and Moorman, M. P. Overview of a system for the computer-assisted operation of a small animal inhalation facility. *Environ. Health Perspect.* 54: 299-310 (1984).
2. Draper, N. R., and Smith, H. *Applied Regression Analysis*. John Wiley and Sons, New York, 1966, pp. 163-216.
3. Searle, S. R. *Linear Models*. John Wiley and Sons, New York, 1971, pp. 82-86.
4. Li, J. C. R. *Statistical Inference I*. Edwards Brothers, Ann Arbor, MI, 1964, pp. 393-399.
5. Searle, S. R. *Matrix Algebra For The Biological Sciences*. John Wiley and Sons, New York, 1966, pp. 225-253.
6. Searle, S. R. *Linear Models*. John Wiley and Sons, New York, 1971, pp. 107-110.